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# Seasonal effects to metallothionein responses to metal exposure in a naturalised population of *Ruditapes philippinarum* in a semi-enclosed estuarine environment

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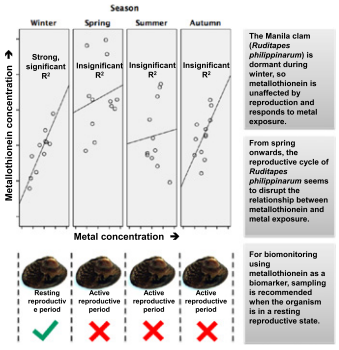
## HIGHLIGHTS

- Seasonal effects on Manila clam metallothionein (MT) at high latitudes were studied.
- Samples were taken throughout 2015 in winter, spring, summer, and autumn.
- Significant positive relationships existed between MT and metals during winter.
- Gametogenesis in spring may override the effect of metals on MT concentrations.
- MT use should be during winter periods, when the Manila clam is not reproducing.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The Manila clam (*Ruditapes philippinarum*), an invasive species in Northern Europe, can be used as a bioindicator of metal pollution. Seasonal effects on metallothionein (MT) production have not been considered in this species at the northernmost extent of its European distribution. This study assesses the annual seasonal effects on MT and metal concentrations in *R. philippinarum* from Poole Harbour, UK. *R. philippinarum* were collected in winter, spring, summer, and autumn throughout 2015, and MT and metal concentrations, as well as biotic and abiotic variables, were quantified. During winter, linear regression analysis showed significant positive relationships between tissue metal and MT concentrations. However, during spring and summer, these relationships were mostly insignificant. MT concentrations during spring had significant positive relationships with tissue and whole weight. Significant positive relationships were also observed between MT and condition index, during summer. During spring and summer, biotic factors seem to override the role of MT as a detoxification mechanism for metal exposure in this species. This is probably due to an increase in MT concentration in spring caused by gametogenesis, associated with increased tissue weight as the gonads expand. A depletion of energy resources, or physical stressors such as heat, may be attributed to the reduced MT production in clams of poor body condition in summer. The evidence from this study suggests that MT may only be a useful biomarker of metal pollution during winter in *R. philippinarum* in the UK. This verifies the natural variability of MT in this species at high latitudes, and highlights the potential and limits to a widely available bioindicator of metal pollution.



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## 1. Introduction

Estuarine environments are often subjected to metal pollution, posing a potentially significant environmental concern. Anthropogenic sources of metals include mining, urbanisation, fuel combustion, and industrial and domestic waste (solid and liquid) disposal. Contaminants entering semi-enclosed systems, such as estuaries, tend to remain close to their sources due to a lack of flushing, therefore posing a long-term risk (Dassenakis et al., 2003). Effects of metal exposure can occur at low concentrations, causing environmental deterioration and can affect the organisms inhabiting these areas (Figueira et al., 2012). Marine organisms, particularly bivalves, offer their use as bioindicator species due to the accumulation of metals, which may reflect concentrations present in the environment. This can be accompanied by the use of biomarkers, such as metallothionein (MT). MTs are non-enzymatic proteins, consisting of thiol groups (sulphur-hydrogen) that bind to metals, preventing oxidative stress to the organism (Amiard et al., 2006). This acts as a detoxification mechanism. An increase in MT concentrations in response to metal contamination has been reported in bivalves by numerous studies (Amiard et al., 2006; Roesijadi, 1994; Serafim and Bebianno, 2007; Bebianno and Langston, 1991; Hamza-Chaffai et al., 2000). Therefore, MT is commonly included in biomonitoring studies and monitoring programmes, such as the Biological Effects and Quality Assurance in Monitoring Programmes (BEQUALM) (Amiard et al., 2006), and in the Natural England suite of assays (Galloway et al., 2008).

Questions remain about the use of MT as a biomarker for metal exposure, as MT may alter independently of metal exposure. This relates to the multifunctional role of MT, such as metabolism of essential metals, and the interaction with natural factors, which may vary seasonally (Geffard et al., 2005). Biotic effects on MT concentrations include mass increases to the digestive gland that may dilute MT concentrations, interferences relating to gametogenesis, physiological condition, and genetic adaptation (Paul-Pont et al., 2010; Baudrimont et al., 1997; Raspor et al., 2004). Abiotic effects on MT concentrations encompass physical parameters such as salinity and temperature (Legras et al., 2000; Hamer et al., 2008; Bocchetti et al., 2008). It is therefore important to establish the seasonal influence of non-metallic controls on MT concentrations in bivalves before they are used for biomonitoring purposes.

The Manila clam (*Ruditapes philippinarum*) was introduced to Poole Harbour, England, in 1988 for aquaculture and is now naturalised (Jensen et al., 2004). Humphreys et al. (2015) document its spread and naturalisation in at least 11 estuaries in southern England, including estuaries with no history of licensed introduction. It is likely *R. philippinarum* will continue to spread throughout the UK, via mechanisms of dispersal such as accidental introduction through fishing from other sites (as well as illegal introductions to establish new fisheries), and if sea surface temperature continues to rise as predicted (supporting breeding and recruitment).

*R. philippinarum* is tolerant of physical stress and pathogens (Tanguy et al., 2008). This is an attribute that has allowed it to spread from the Indian-Pacific region to Atlantic and Mediterranean coasts (Delgado and Perez-Camacho, 2007). Its wide-ranging presence offers an opportunity to evaluate its potential as a widely available bioindicator species. However, seasonal effects on MT concentration in species at their limit of distribution are unknown. MT response to metal exposure in *R. philippinarum* has not been studied as far north as the UK, or Northern Europe, nor as a general bioindicator species for metal pollution. Studies have found that *R. philippinarum* produces MT in response to metal exposure (Won et al., 2012; Figueira et al., 2012; Wang et al., 2011). There is also evidence that seasonal effects, such as reproductive cycle or temperature, can influence the apparent MT response to metal exposure in *R. philippinarum* (Bocchetti et al., 2008; Moschino et al., 2012). Further analysis of seasonal effects on MT response in this species is needed before it can be implemented in monitoring programmes as a reliable biomarker.

Metal accumulation is particularly relevant in this species as it is an important shellfishery resource in Poole Harbour, and in much of Europe, and is consumed by the human population. Holes Bay in Poole Harbour is a closed fishery due to environmental contamination (Aly et al., 2013). Although regulatory enforcement largely prevents illegal fishing, there is still a risk of human consumption of shellfish from this area. Metal contamination is not always quantified in shellfish intended for consumption despite other pollutant analysis, such as microbial contaminants (Freitas et al., 2012). Hence, currently, the amount of metals within the organisms from closed fishery areas of Poole Harbour, and indeed other areas fished commercially, is unknown. It is important to continually monitor metals in marine invertebrates in order to manage the risk to human health from seafood consumption (Figueira and Freitas, 2013). Furthermore, Poole Harbour holds a number of statutory designations to protect the natural environment as it is recognised as being of international important for protected habitats and species, such as overwintering waterbirds on intertidal mud-flats (Natural England, 2015). Designations include a Special Protection Area (SPA), a Ramsar site and a Site of Special Scientific Interest (SSSI), which includes Holes Bay.

This study aimed to evaluate the potential of *R. philippinarum* as a MT biomarker species at the northernmost extent of its distribution for the first time. It also aimed to evaluate the risk of its consumption relating to tissue metal concentrations.

## 2. Methodology

### 2.1. Field sampling

Four sites within Poole Harbour were studied: Wareham Channel, Arne Bay, Holes Bay and Holes Bay (north) (Fig. 1). *R. philippinarum* of length between 32 mm and 45 mm were dredged from the seabed in each of these sites, using a pump scoop (steel scooped cage designed to be dragged along seabed, sifting mud and collecting invertebrates) from a small survey vessel. Four samplings were conducted throughout 2015 in January, April, August, and October. These are referred to as winter, spring, summer, and autumn samples respectively. Sea surface temperature, pH, dissolved oxygen and conductivity were also measured at each site during sampling. Three replicate sediment samples were collected to test metal concentrations from each site using plastic piping (to prevent metal contamination) during the autumn sample (October). The upper layer of approximately 10 cm was sampled, where clams reside. The pre-treatment of organisms before analysis was as advised by Oaten et al. (2015). Care was taken to prevent trace metal contamination by using a ceramic blade for dissection, where possible. Three replicates of at least six individuals were dissected for each sample into digestive gland, gills, and remaining tissue. During dissection, whole weight (including shell), and tissue weight was measured. This was used to calculate condition index (soft tissue weight (g)/whole weight (g) including shell and pallial liquid) (Mourgaud et al., 2002). Whole tissue concentrations (of MT and metals) were calculated from digestive gland, gill, and remaining tissue concentration based on the ratio of component weights.

### 2.2. MT analysis

A modified spectrophotometric method, as described by Viarengo et al. (1997) with modifications by Aly et al. (2014), was used to measure MTs in *R. philippinarum*. This is a sensitive, time saving, and low cost technique that has been inter-calibrated and standardized by a number of laboratories (UNEP/RAMOGE, 1999; Zorita et al., 2005). Frozen samples of digestive gland, gill, and remaining tissue were first homogenised using a ceramic pestle and mortar. An accurately weighed sample of approximately 1 g was taken from three replicates. Tissue samples were further homogenised with 3 ml homogenising buffer of 0.5 M sucrose, 20 mM Tris–HCl (pH 8.6), 0.006 mM leupeptin, 0.5 mM

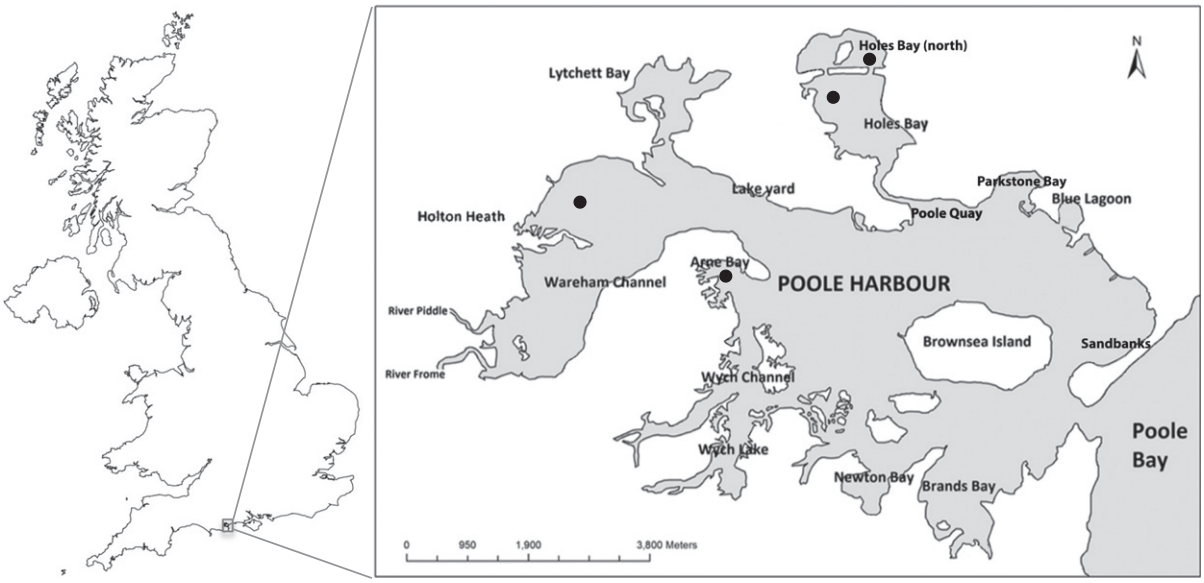


**Fig. 1.** Site map of Poole Harbour, UK, and sampling locations.

phenylmethylsulfonyl fluoride (PMSF), and 0.01% B-mercaptoethanol, then centrifuged for 30 min at 24,600 ×*g* (times gravity) at 4 °C. The supernatant solution was purified with 1.05 ml annular grade cold ethanol (−20 °C) and 80 μl chloroform (per 1 ml of supernatant) and centrifuged for 10 min at 6000 ×*g* at 4 °C. Supernatant solution was then collected and 40 μl of 35% hydrochloric acid (HCl) and three times the supernatant volume of annular grade cold ethanol was added and left to allow the proteins to denature for at least 1 h at −20 °C. This was centrifuged at 6000 ×*g* for 10 min and the pellet saved. The pellet was washed with the previously described buffer (without B-mercaptoethanol, leupeptin, and PMSF), annular grade cold ethanol and 80 μl chloroform (87:12:1 *v*/v) and centrifuged for 10 min at 6000 ×*g*. The supernatant was discarded and the pellet dried with nitrogen gas. The pellet was resuspended with 150 μl 0.25 M sodium chloride (NaCl) and 150 μl 1 N HCl with 4 mM EDTA. After resuspension, 4.2 ml of a solution containing 2 M NaCl, 0.43 mM DTNB and 0.2 M sodium phosphate ($NaH_2PO_4$) (pH 8.0) was added, and mixed by centrifugation at 3000 ×*g* for 5 min. The absorbance was read at 412 nm against standard solutions of reduced glutathione (GSH) using a UV–visible spectrophotometer (Cecil 3000 series). Results are reported as μg/g (wet weight).

### 2.3. Tissue metal analysis

The metals determined were Cr, Fe, Ni, Cu, Zn, Ag, Cd, Sn, Pb, and the metalloid As, due to a combination of their presence in Poole Harbour and the potential for bioaccumulation (Aly et al., 2013). Frozen homogenised *R. philippinarum* digestive gland, gill, remaining tissue replicates (as per first step in MT analysis) were freeze-dried for three days. Accurately weighed samples of approximately 10 mg of dried, ground sample were placed in 7 ml Teflon sealable pots. Blank samples consisting of empty Teflon pots were also prepared for analytical quality assurance. 1 ml of a 3:1 *v*/v solution of trace metal grade, redistilled 37% HCl and 68% nitric acid ($HNO_3$) (Aqua Regia) was added. Sealed pots were then heated at 60 °C for 4 h, and then heated at 120 °C for a further 12 h on a hot plate. Samples were dried and 1 ml of $HNO_3$ was added and heated to 120 °C for 12 h. Samples were then dried again and 300 μl of trace metal grade hydrogen peroxide ($H_2O_2$) was added to samples, and heated unsealed at 120 °C until fully reacted. Samples were dried and resuspended with 3% $HNO_3$, containing 5 ppb In/Re and 20 ppb Be as internal standards to correct for matrix effects and instrument drift for analytical quality assurance. Samples were subsequently emptied and washed into vials, and completed to a 4000-fold dilution with 3% $HNO_3$ containing 5 ppb In/Re and 20 ppb Be, via a two-step process. At each stage of dilution and completion, empty and full vials were weighed to calculate accurate dilution factors. Analysis by inductively coupled plasma mass spectrometry (ICP–MS) was carried out. Five, matrix matched, standard solutions containing Cr, Fe, Ni, Cu, Zn, As, Ag, Cd, Sn, and Pb at known concentrations were used to calibrate and quantify metal concentrations of the samples. A mussel reference material (European Reference Materials – CE278k) was measured as a bivalve comparator and concentrations were adjusted according to the recovery rate (Table 1). Limits of detection (LOD) have also been calculated as three times the standard deviation of 20 blank sample concentrations above the mean blank sample concentration (Analytical Methods Committee, 1987; Armbruster and Pry, 2008) (Table 1).

### 2.4. Sediment metal analysis

The same metals as analysed in organism tissue were analysed in sediments. Samples were freeze-dried and sieved using a 0.25 mm, non-metallic sieve, to remove large stones or shells. This was as advised by Hubner and Haslam (2011), and based on a particle size distribution analysis confirming all sediments had similar grain sizes, and <1% of grains were >0.25 mm in diameter. Accurately weighed samples of approximately 50 mg were placed in 20 ml Teflon sealable pots. Blank samples consisting of empty Teflon pots were also prepared. 1 ml of trace metal grade, redistilled $HNO_3$ was added to the samples, followed by 2 ml of trace metal grade, redistilled hydrofluoric acid (HF). Sealed pots were heated at 130 °C for 12 h on a hot plate. Samples were then dried, 2 ml of trace metal grade, redistilled 6 M HCl was added, and left at 130 °C for 12 h on a hot plate. Samples were subsequently dried down; 2 ml of $HNO_3$ was added, followed by 1 ml of $H_2O_2$, which was repeated until the samples were clear. Samples were then dried and resuspended with 3% $HNO_3$, containing 5 ppb In/Re and 20 ppb Be as internal standards to correct for matrix effects and instrument drift, and completed and measured as per tissue analysis. Results for both sediment and tissue metal concentrations are reported as μg/g (dry weight) relative to original sample weight taken.

Canadian sediment quality guidelines (CSQGs), which are cautiously recommended by the Centre for Environment Fisheries Aquaculture Science (CEFAS) in the absence of statutory standards in the UK (Langston et al., 2003b), were used to assess the magnitude of sediment metal pollution in Poole Harbour. CSQGs consist of threshold effect levels (TELs) at which effects may be observed in some sensitive species, and

**Table 1**
Recovery values (%) for metals in mussel reference material (European Reference Materials – CE278k) averaged across seasons, and limits of detection (calculated as three times the standard deviation of 20 blank sample concentrations above the mean blank sample concentration).

| Metal | Cr | Fe | Ni | Cu | Zn | As | Ag | Cd | Sn | Pb |
|---|---|---|---|---|---|---|---|---|---|---|
| Recovery rate (%) | 98.1 | 93.85 | 86.32 | 85.86 | 98.40 | 78.49 | – | 91.56 | – | 89.56 |
| Limits of detection (μg/g) | 0.076 | 3.817 | 0.039 | 0.038 | 2.453 | 0.078 | 0.009 | 0.002 | 0.019 | 0.023 |

probable effect levels (PELs) at which adverse effects are likely in a wide range of organisms (Hubner et al., 2009).

### 2.5. Statistical analyses

All statistical analyses were completed using IBM SPSS Statistic v21. Tests for normality (Shapiro-Wilk) and homogeneity of variance (Levene's test) were completed and data was tested parametrically (one-way ANOVA) or non-parametrically (Kruskal-Wallis test), accordingly. Linear regression was used to determine the effects of metal exposure and biotic and abiotic variables on MT concentrations in *R. philippinarum*. Statistical significance was established at $P = 0.05$.

## 3. Results

### 3.1. Spatial variation of MT and metal concentrations, by season

In **winter**, MT concentrations were highest in *R. philippinarum* collected from Holes Bay (north), closely followed by Holes Bay (Table 2). *R. philippinarum* from Arne Bay on the west side of Poole Harbour had the lowest concentration of MT. Digestive gland MT concentrations were significantly higher in Holes Bay (north) compared to the Wareham Channel and Arne Bay (*post-hoc* Scheffe, $P = 0.024$, $P = 0.019$, respectively). Gill MT concentrations were significantly higher in Holes Bay (north) compared to Arne Bay (Kruskal-Wallis, pairwise comparison, $P = 0.028$). Whole tissue MT concentrations were

**Table 2**
Concentrations (μg/g) of metallothionein (MT), Cr, Fe, Ni, Cu, Zn, As, Ag, Cd, Sn, Pb in digestive gland (DG), gills (G), and whole tissue (WT) from *R. philippinarum* from Wareham Channel (WC), Arne Bay (AB), Holes Bay (HB), and Holes Bay (north) (HB (n)) in Poole Harbour throughout each season in 2015.

| | | Winter | | | Spring | | | Summer | | | Autumn | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DG | G | WT | DG | G | WT | DG | G | WT | DG | G | WT |
| MT | WC | 23.74 | 9.42 | 18.33 | 34.11 | 13.89 | 29.16 | 24.19 | 10.65 | 19.49 | 26.44 | 7.28 | 17.67 |
| | AB | 23.09 | 7.15 | 15.92 | 27.75 | 10.02 | 20.87 | 22.66 | 11.19 | 20.38 | 28.12 | 11.97 | 20.79 |
| | HB | 26.70 | 11.62 | 20.39 | 40.91 | 15.94 | 31.40 | 29.75 | 11.78 | 27.93 | 32.84 | 10.63 | 21.67 |
| | HB (n) | 37.83 | 15.34 | 24.77 | 35.63 | 10.60 | 25.65 | 19.11 | 7.31 | 15.86 | 25.45 | 12.51 | 25.19 |
| Cr | WC | 2.79 | 0.84 | 1.63 | 2.12 | 0.88 | 1.77 | 2.48 | 1.84 | 1.99 | 4.14 | 1.26 | 3.09 |
| | AB | 1.28 | 0.77 | 1.02 | 2.48 | 0.98 | 3.12 | 3.18 | 1.72 | 2.40 | 2.89 | 1.03 | 1.97 |
| | HB | 3.48 | 0.87 | 2.16 | 4.84 | 1.61 | 3.67 | 2.71 | 0.76 | 1.89 | 3.49 | 0.92 | 1.98 |
| | HB (n) | 3.71 | 1.00 | 2.04 | 5.73 | 1.58 | 4.35 | 2.77 | 0.95 | 2.20 | 3.37 | 1.07 | 2.38 |
| Fe | WC | 1248 | 608.4 | 992.4 | 1412 | 634.7 | 1172 | 2505 | 1051 | 1770 | 3692 | 715.8 | 2260 |
| | AB | 1338 | 1095 | 1086 | 1618 | 971.1 | 1958 | 3345 | 1282 | 2470 | 2734 | 1739 | 2202 |
| | HB | 2835 | 1091 | 1996 | 3007 | 1336 | 2553 | 2821 | 963.1 | 2430 | 3440 | 1400 | 2266 |
| | HB (n) | 3051 | 1423 | 1805 | 3467 | 955.3 | 2427 | 2873 | 1443 | 2493 | 3316 | 1106 | 2473 |
| Ni | WC | 4.11 | 7.78 | 5.46 | 4.18 | 7.64 | 5.96 | 4.20 | 8.46 | 5.76 | 5.28 | 7.32 | 6.42 |
| | AB | 3.18 | 5.73 | 4.00 | 5.97 | 7.04 | 7.39 | 4.46 | 7.49 | 5.86 | 4.35 | 6.47 | 5.21 |
| | HB | 5.95 | 5.37 | 5.73 | 6.86 | 7.99 | 7.44 | 3.74 | 4.93 | 4.82 | 4.64 | 5.37 | 4.90 |
| | HB (n) | 5.96 | 6.42 | 6.27 | 8.35 | 7.47 | 8.54 | 4.67 | 6.27 | 5.63 | 4.48 | 5.62 | 5.50 |
| Cu | WC | 11.63 | 7.12 | 8.64 | 11.44 | 5.62 | 7.85 | 12.52 | 6.95 | 9.13 | 12.55 | 6.37 | 8.80 |
| | AB | 9.31 | 6.28 | 6.85 | 10.54 | 5.62 | 7.92 | 11.60 | 6.00 | 9.09 | 9.57 | 7.08 | 7.95 |
| | HB | 18.72 | 6.19 | 10.80 | 16.83 | 6.00 | 10.37 | 16.74 | 6.12 | 11.74 | 16.76 | 10.48 | 11.71 |
| | HB (n) | 18.89 | 6.83 | 10.45 | 17.60 | 5.87 | 11.10 | 17.80 | 7.69 | 13.59 | 18.34 | 5.97 | 12.57 |
| Zn | WC | 76.51 | 118.5 | 92.93 | 77.71 | 115.7 | 101.5 | 80.09 | 144.6 | 90.56 | 76.34 | 90.07 | 79.23 |
| | AB | 77.55 | 97.72 | 79.87 | 85.87 | 97.37 | 99.10 | 79.13 | 117.8 | 88.17 | 68.55 | 90.06 | 74.10 |
| | HB | 113.6 | 98.82 | 106.9 | 120.4 | 141.8 | 134.4 | 95.29 | 118.6 | 109.8 | 87.78 | 101.3 | 96.80 |
| | HB (n) | 107.4 | 107.8 | 101.6 | 105.5 | 114.6 | 114.2 | 96.79 | 121.4 | 118.6 | 82.33 | 106.8 | 100.8 |
| As | WC | 16.92 | 13.80 | 15.78 | 15.02 | 14.67 | 14.51 | 14.43 | 17.60 | 15.45 | 21.35 | 18.91 | 19.31 |
| | AB | 22.82 | 18.42 | 19.85 | 20.89 | 18.40 | 19.47 | 19.23 | 22.46 | 21.05 | 17.81 | 19.72 | 17.67 |
| | HB | 26.64 | 15.75 | 20.70 | 22.24 | 22.46 | 20.62 | 16.57 | 21.40 | 19.32 | 21.39 | 20.88 | 20.93 |
| | HB (n) | 28.72 | 17.31 | 20.97 | 17.75 | 16.88 | 16.15 | 16.61 | 20.58 | 17.96 | 17.85 | 18.73 | 18.42 |
| Ag | WC | 1.06 | 0.20 | 0.70 | 0.59 | 0.18 | 0.47 | 0.92 | 0.23 | 0.74 | 1.39 | 0.12 | 0.98 |
| | AB | 0.27 | 0.09 | 0.17 | 1.01 | 0.06 | 0.55 | 0.49 | 0.11 | 0.48 | 0.23 | 0.08 | 0.25 |
| | HB | 3.06 | 0.15 | 1.41 | 3.72 | 0.11 | 1.81 | 1.57 | 0.13 | 1.32 | 2.26 | 0.23 | 1.29 |
| | HB (n) | 2.49 | 1.02 | 1.83 | 3.65 | 0.19 | 2.31 | 4.89 | 0.45 | 3.41 | 4.63 | 0.24 | 3.53 |
| Cd | WC | 0.50 | 0.71 | 1.08 | 0.56 | 0.59 | 0.87 | 0.73 | 0.88 | 0.89 | 0.79 | 0.52 | 1.17 |
| | AB | 0.66 | 0.24 | 0.52 | 0.68 | 0.32 | 0.77 | 0.77 | 0.28 | 0.67 | 0.40 | 0.44 | 0.52 |
| | HB | 1.09 | 0.27 | 0.92 | 0.85 | 0.32 | 0.88 | 0.40 | 0.30 | 0.72 | 0.59 | 0.39 | 0.78 |
| | HB (n) | 0.78 | 0.57 | 0.88 | 0.99 | 0.41 | 0.93 | 0.54 | 0.56 | 0.76 | 0.45 | 0.34 | 0.90 |
| Sn | WC | 0.33 | 0.07 | 0.16 | 0.15 | 0.06 | 0.12 | 0.30 | 0.27 | 0.21 | 0.23 | 0.07 | 0.17 |
| | AB | 0.11 | 0.07 | 0.08 | 0.13 | 0.05 | 0.14 | 0.23 | 0.11 | 0.17 | 0.16 | 0.06 | 0.11 |
| | HB | 0.32 | 0.13 | 0.23 | 0.35 | 0.13 | 0.31 | 0.35 | 0.10 | 0.27 | 0.30 | 0.13 | 0.20 |
| | HB (n) | 0.37 | 0.11 | 0.25 | 0.42 | 0.13 | 0.33 | 0.34 | 0.13 | 0.31 | 0.32 | 0.09 | 0.24 |
| Pb | WC | 1.52 | 0.54 | 1.26 | 2.30 | 0.59 | 1.76 | 4.39 | 2.08 | 2.92 | 4.97 | 0.64 | 3.15 |
| | AB | 1.74 | 0.57 | 1.21 | 2.08 | 0.85 | 2.07 | 4.80 | 1.11 | 3.11 | 3.52 | 1.52 | 2.58 |
| | HB | 5.55 | 0.83 | 3.34 | 6.08 | 0.73 | 4.20 | 5.84 | 0.94 | 4.09 | 6.26 | 0.92 | 4.03 |
| | HB (n) | 5.10 | 2.00 | 3.42 | 7.25 | 0.85 | 4.81 | 5.66 | 1.27 | 4.41 | 5.31 | 0.84 | 4.43 |

significantly higher in Holes Bay (north) compared to the Wareham Channel and Arne Bay (*post-hoc* Scheffe, $P = 0.033$, $P = 0.006$, respectively).

In **spring**, MT concentrations in *R. philippinarum* from Holes Bay (north) decreased whilst concentrations in *R. philippinarum* from Wareham, Arne Bay, and Holes Bay increased, leaving Holes Bay MT concentrations to be highest. MT concentrations in the digestive gland were significantly lower in Arne Bay than in the Wareham Channel, Holes Bay, and Holes Bay (north), and MT concentrations in Holes Bay were significantly higher than in the Wareham Channel (*post-hoc* Scheffe, $P = 0.041$, $P = 0.001$, $P = 0.013$, $P = 0.029$, respectively). Gill MT concentrations were significantly higher in Holes Bay compared to Arne Bay and Holes Bay (north) (*post-hoc* Scheffe, $P = 0.006$, $P = 0.011$, respectively). Whole tissue MT concentrations were significantly lower in Arne Bay compared to the Wareham Channel and Holes Bay (Kruskal-Wallis, pairwise comparison, $P = 0.013$, $P = 0.009$, respectively).

In **summer**, *R. philippinarum* from Holes Bay (north) had the lowest concentrations of MT, whilst Holes Bay MT concentrations remained the highest. Whole tissue MT concentrations were significantly higher in Holes Bay compared to the Wareham Channel, Arne Bay, and the Holes Bay (north) (*post-hoc* Scheffe, $P = 0.003$, $P = 0.005$, $P < 0.001$, respectively).

This pattern remained similar in **autumn**; however MT concentrations increased in *R. philippinarum* from Holes Bay (north), and were significantly higher than in the Wareham Channel and Arne Bay in whole tissue (*post-hoc* Scheffe, $P = 0.003$, $P = 0.05$, respectively). MT concentrations in gills were significantly lower in the Wareham Channel compared to Arne Bay and Holes Bay (north) (*post-hoc* Scheffe, $P = 0.023$, $P = 0.012$, respectively).

For tissue metal concentrations (Table 2) in *R. philippinarum*, Cu, Zn, Ag, Sn, and Pb exhibited a similar pattern in each season: highest concentrations in Holes Bay (north) and Holes Bay, and lowest concentrations in Wareham Channel and Arne Bay. This pattern also generally existed for Cr, Fe, Ni, As, and Cd during winter and spring. However, during summer and autumn, patterns of these metal concentrations altered across sites.

Sediment metal concentrations were significantly higher in Holes Bay (north), followed by Holes Bay, Arne Bay, and Wareham Channel for Cr, Cu, Zn, Ag, Cd, Sn, and Pb (Fig. 2). For Fe and Ni, sediment concentrations were highest in Holes Bay. For As, sediment concentrations were highest in Arne Bay, followed by Holes Bay, Holes Bay (north), and Wareham Channel.

### 3.2. Between season variation in MT response to metal exposure

MT concentrations in *R. philippinarum* varied between each season with generally higher concentrations in spring, apart from in Holes Bay (north) where MT concentrations were lowest in summer. **Wareham Channel** digestive gland MT was significantly higher in spring compared to winter (Kruskal-Wallis, pairwise comparison, $P = 0.017$) and summer (Kruskal-Wallis, pairwise comparison, $P = 0.013$). Gill MT was significantly higher in spring compared to winter (*post-hoc* Scheffe, $P = 0.017$) and autumn (*post-hoc* Scheffe, $P = 0.002$). **Arne Bay** gill MT was significantly lower in winter compared to autumn (*post-hoc* Scheffe, $P = 0.035$). **Holes Bay** digestive gland MT was significantly higher in spring compared to winter (*post-hoc* Scheffe, $P = 0.028$). Gill MT in spring was significantly higher than winter, summer, and autumn (*post-hoc* Scheffe, $P = 0.019$, $P = 0.023$, $P = 0.006$, respectively). Whole tissue MT in winter was significantly lower than in spring (Kruskal-Wallis, pairwise comparisons, $P = 0.007$), and summer (Kruskal-Wallis, pairwise comparisons, $P = 0.017$). **Holes Bay** (**north**) digestive gland MT in summer was significantly lower than in winter (Kruskal-Wallis, pairwise comparisons, $P = 0.007$), and spring (Kruskal-Wallis, pairwise comparisons, $P = 0.017$). Similarly, gill MT was significantly lower in summer compared to winter (Kruskal-Wallis, pairwise comparisons, $P = 0.009$), and autumn (Kruskal-Wallis, pairwise comparisons, $P = 0.031$).

Linear regression analysis was performed to determine the importance of metal exposure to MT induction across seasons, which was the focus of this study. In winter many significant positive relationships existed between MT and metal concentrations in each tissue, though fewer existed in gills compared to digestive gland and whole tissue (Table 3). However in spring, summer, and autumn, fewer significant positive relationships existed in whole tissue (Fig. 3). This was also the case in digestive gland and gill tissue, and between sediment metal concentrations and tissue MT concentrations (analyses not reported).

Linear regression analyses with other biotic variables were examined to determine their influence. Statistical data and the strength of relationships are noted within parentheses. Tissue weight had significant positive relationships with MT concentrations during spring in digestive gland ($R^2 = 0.519$ (strong), $P = 0.008$), and whole tissue ($R^2 = 0.577$ (strong), $P = 0.004$), and during autumn in whole tissue ($R^2 = 0.830$ (very strong), $P < 0.001$) (Fig. 4). Whole weight also had some significant positive relationships with MT concentrations during spring in digestive gland ($R^2 = 0.723$ (strong), $P < 0.001$), and whole tissue ($R^2 = 0.491$ (strong), $P = 0.011$), and during autumn in whole tissue ($R^2 = 0.736$ (strong), $P < 0.001$) (Fig. 5). Condition index showed a positive relationship with MT concentrations during summer in digestive gland ($R^2 = 0.374$ (moderate), $P = 0.035$), and whole tissue ($R^2 = 0.368$ (moderate), $P = 0.037$) (Fig. 6). Gill MT also showed significant positive relationships between: tissue weight during spring ($R^2 = 0.477$ (moderate), $P = 0.013$) and autumn ($R^2 = 0.453$ (moderate), $P = 0.017$); whole weight during spring ($R^2 = 0.520$ (strong), $P = 0.008$), and autumn ($R^2 = 0.347$ (moderate), $P = 0.044$); and condition index during spring ($R^2 = 0.349$ (moderate), $P = 0.043$).

### 3.3. Localised variation in MT response

Variation in MT response between sites was analysed for completeness. Linear regression analysis showed significant positive relationships between MT and metal concentrations at all sites in *R. philippinarum*, except for the **Wareham Channel**. Here, MT concentrations were also not affected by tissue weight, whole weight, or condition index.

*R. philippinarum* from **Arne Bay** showed a negative relationship between whole weight and MT concentration in gills ($R^2 = 0.498$, $P = 0.01$), and whole tissue ($R^2 = 0.547$ (strong), $P = 0.006$). A positive relationship existed between MT and condition index in gills ($R^2 = 0.496$ (strong), $P = 0.011$).

Unlike Arne Bay, *R. philippinarum* from **Holes Bay** showed positive relationships between whole weight and MT concentration in gills ($R^2 = 0.641$ (strong), $P = 0.002$), and in whole tissue ($R^2 = 0.448$ (moderate), $P = 0.017$). There were also positive relationships between MT concentration and tissue weight in gills ($R^2 = 0.507$ (strong), $P = 0.009$), and whole tissue ($R^2 = 0.645$ (strong), $P = 0.002$).

*R. philippinarum* from **Holes Bay** (**north**) showed negative relationships between MT concentration in the digestive gland and whole weight ($R^2 = 0.763$ (strong), $P < 0.001$), and tissue weight ($R^2 = 0.593$ (strong), $P = 0.003$).

The relationship between abiotic factors and MT concentration was also examined at each site using linear regression analysis (Tables 4 and 5). Consistent relationships were not observed across sites. Unlike all other sites, negative relationships existed between temperature and MT concentrations in the digestive gland ($R^2 = 0.592$ (strong), $P = 0.003$), gills ($R^2 = 0.532$ (strong), $P = 0.007$), and whole tissue ($R^2 = 0.452$ (moderate), $P = 0.017$), in *R. philippinarum* from Holes Bay (north).

## 4. Discussion

### 4.1. Metal contamination in Poole Harbour

Sediment metal concentrations were mostly highest in Holes Bay (north). Tissue metal concentrations in *R. philippinarum* were also generally highest in Holes Bay (north), but some metal concentrations

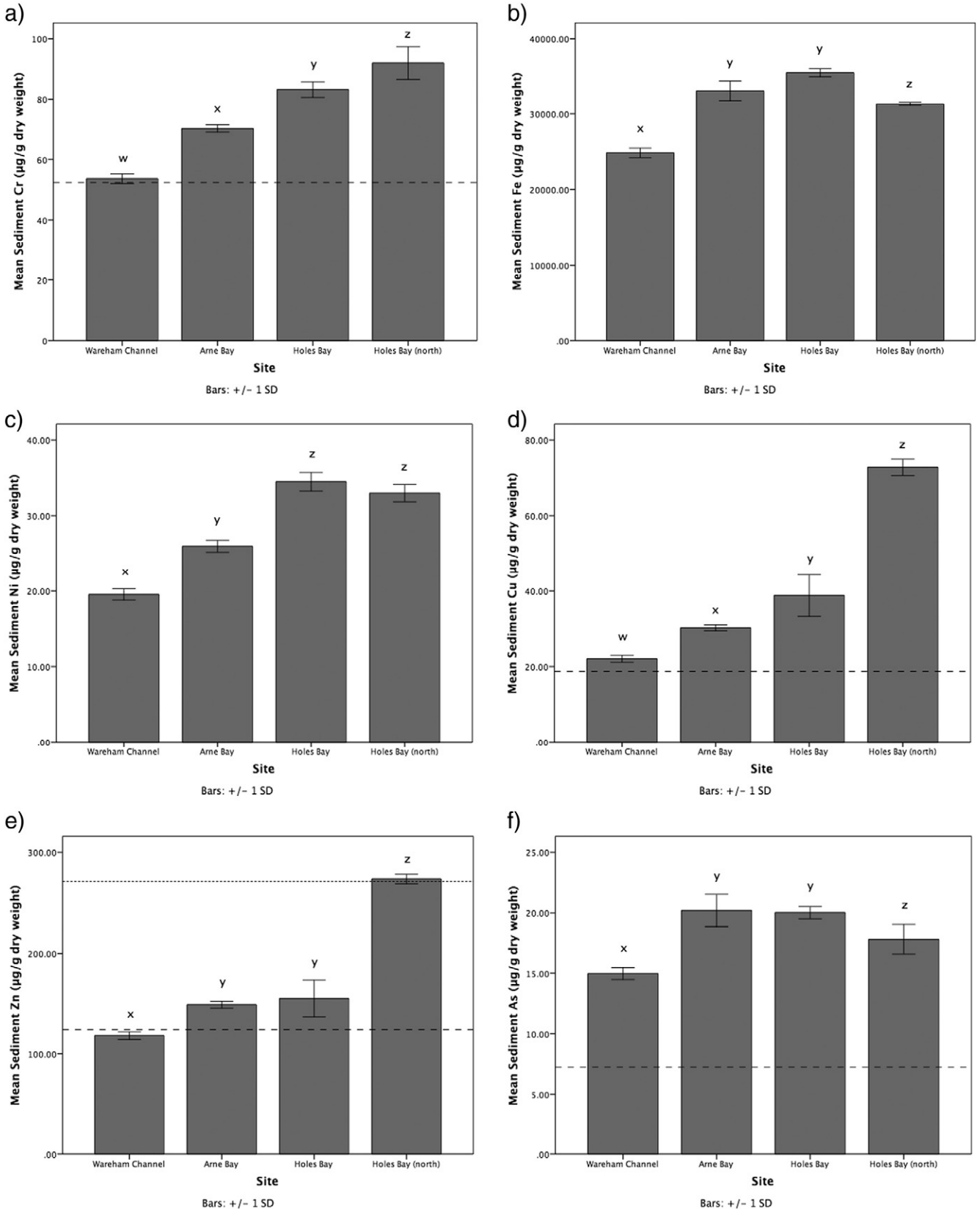


**Fig. 2.** Concentrations (µg/g) of a) Cr, b) Fe, c) Ni, d) Cu, e) Zn, and f) As, g) Ag, h) Cd, i) Sn, and j) Pb in sediments from Poole Harbour, with standard deviation (SD) (n = 3) (dashed lines indicate the Threshold Effect Level (TEL) and dotted line indicates the Probable Effect level (PEL) (Hubner et al., 2009)). Different letters indicate significant differences. Concentrations (µg/g) of a) Cr, b) Fe, c) Ni, d) Cu, e) Zn, and f) As, g) Ag, h) Cd, i) Sn, and j) Pb in sediments from Poole Harbour, with standard deviation (SD) ($n = 3$) (dashed lines indicate the Threshold Effect Level (TEL) and dotted line indicates the Probable Effect level (PEL) (Hubner et al., 2009)). Different letters indicate significant differences.

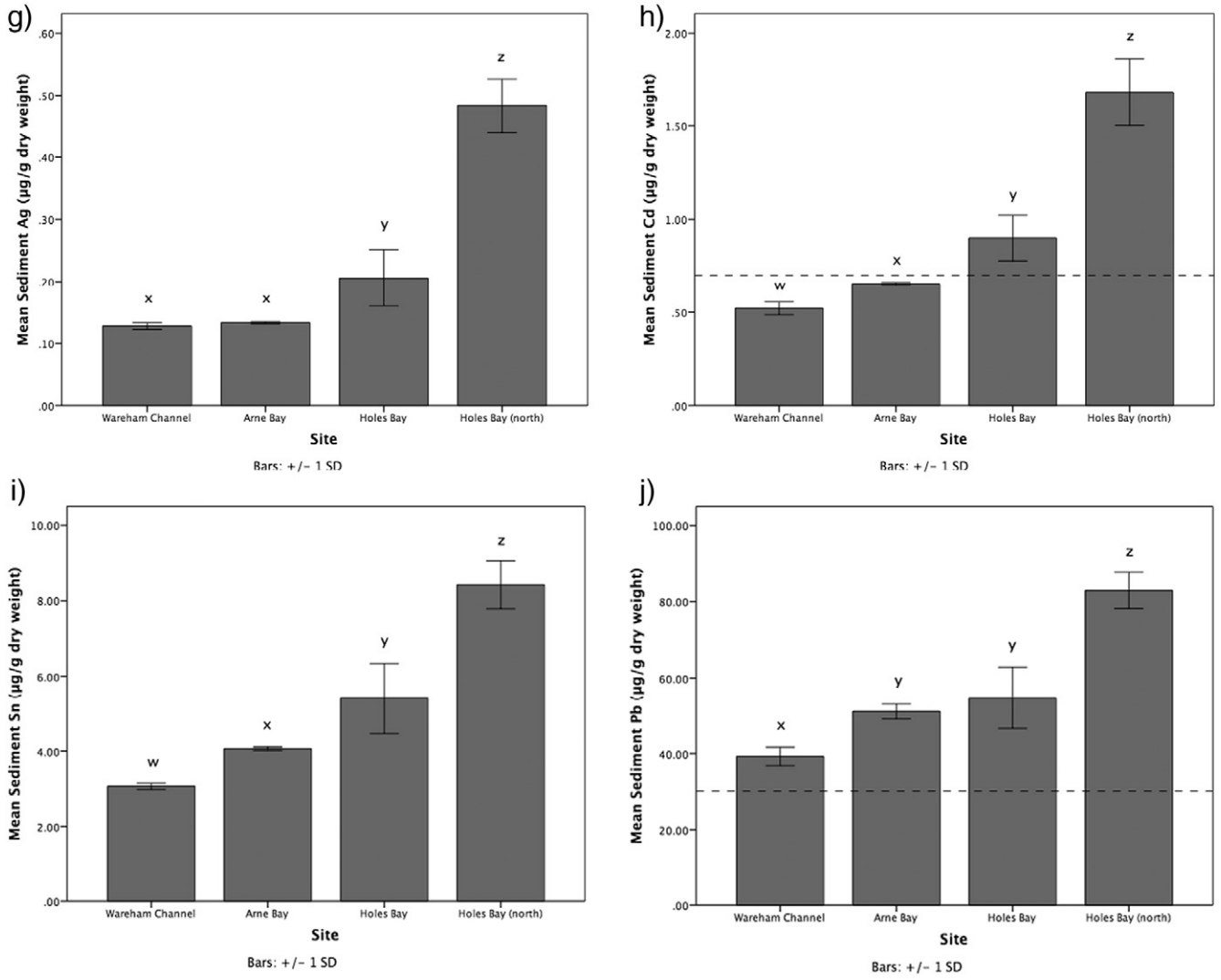


**Fig. 2** (*continued*).

varied seasonally. The magnitude of metal contamination found in the present study is consistent with previous work (Aly et al., 2013; Langston et al., 2003b). Metal pollution in Holes Bay is probably partly due to historic contamination that resides within the sediments due to low water circulation in this system. This occurrence may be more prevalent in Holes Bay (north), where flushing is even more restricted. A coal-fired power station, which was closed and demolished in 1993, was situated on the southwest shore of Holes Bay. This may have been a source of various metals such as Pb, Sn, and Ni. Another historic source of metals, which may still be present in the environment, is the Merck chemical plant and electroplating works, which stopped operations in 1998 (Langston et al., 2003b). Cr, As, Cu, Cd, Pb, Hg, Ni, and Zn were discharged into Holes Bay from the plant during its operation (Langston et al., 2003b). There was also a major fire at this site in 1988, which may have led to polluted water entering Holes Bay (Hansard, 1988). The largest sources of contamination to the area, both historically and currently, include a large concentration of combined sewer overflows (CSOs) and a sewage treatment works (STW),

**Table 3**
Linear regression between tissue metal concentrations and MT concentrations in *R. philippinarum* in each tissue, during winter ($P = 0.05$, significant results are marked with *).

| | Digestive gland MT | | Gill MT | | Whole tissue MT | |
|---|---|---|---|---|---|---|
| | $R^2$ | Sig. | $R^2$ | Sig. | $R^2$ | Sig. |
| Cr | 0.391 | 0.03* | 0.728 | <0.001* | 0.608 | 0.005* |
| Fe | 0.528 | 0.007* | 0.564 | 0.005* | 0.447 | 0.024* |
| Ni | 0.391 | 0.03* | 0.031 | 0.583 | 0.384 | 0.042* |
| Cu | 0.426 | 0.021* | 0.004 | 0.846 | 0.63 | 0.004* |
| Zn | 0.398 | 0.028* | 0 | 0.963 | 0.545 | 0.009* |
| As | 0.284 | 0.074 | 0.05 | 0.486 | 0.116 | 0.306 |
| Ag | 0.243 | 0.104 | 0.787 | <0.001* | 0.581 | 0.006* |
| Cd | 0.147 | 0.219 | 0.261 | 0.089 | 0.146 | 0.246 |
| Sn | 0.203 | 0.141 | 0.446 | 0.018* | 0.774 | <0.001* |
| Pb | 0.422 | 0.022* | 0.81 | <0.001* | 0.523 | 0.012* |

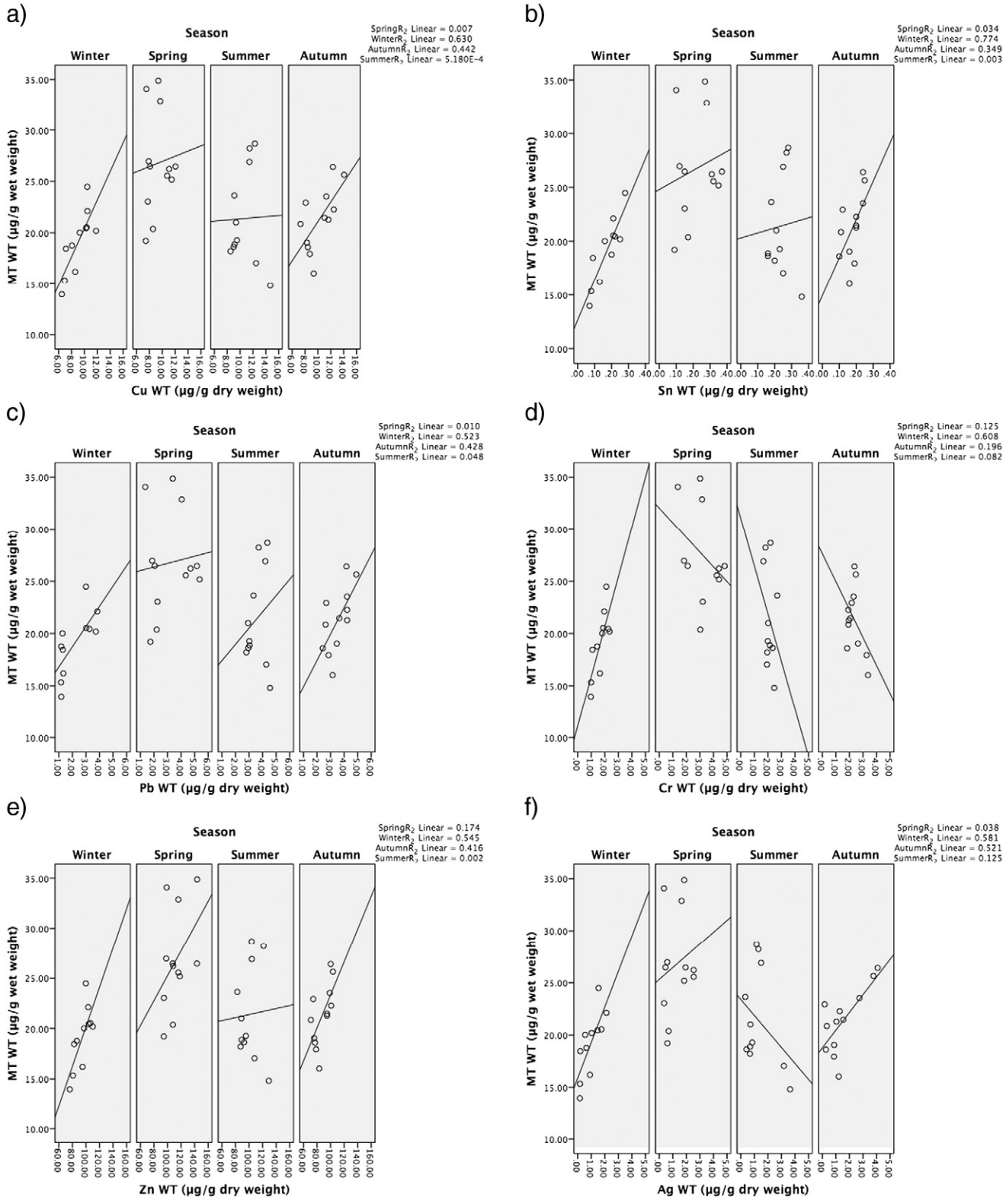


**Fig. 3.** Linear regression between concentrations of MT and a) Cu, b) Sn, c) Pb, d) Cr, e) Zn, and f) Ag in whole tissue (WT) from *R. philippinarum* from Poole Harbour across seasons in 2015.

owned by Wessex Water, that discharge into the northeast corner of Holes Bay (north) (Langston et al., 2003b).

Most sites in Poole Harbour exceed CSQGs. Sediment concentrations of Cr, Cu, As, and Pb exceed TELs in all sites in Poole Harbour. Zn and Cd sediment concentrations exceed TELs in all but the Wareham Channel, and the Wareham Channel and Arne Bay, respectively. Zn concentrations also exceed PELs in Holes Bay (north). However, European Commission (EC) Regulation 1881/2006 sets maximum levels for certain contaminants in foodstuffs. The maximum concentration of Pb and Cd permitted in bivalve molluscs is 1.5 µg/g (wet weight) and 1 µg/g (wet weight), respectively. These can be converted from wet weight to dry weight concentrations using a conversion factor of 7, assuming moisture content of 85% (Dincer, 2006; Pan and Wang, 2012; Zhao et al., 2013). These equate to 10 µg/g (dry weight) and 7 µg/g

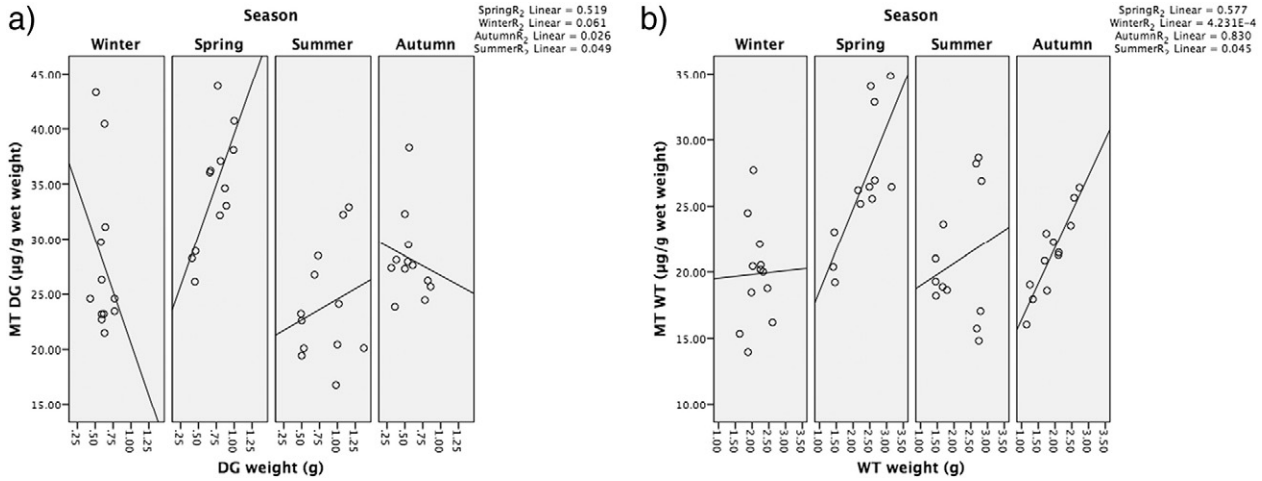


**Fig. 4.** Linear regression between MT concentrations in *R. philippinarum* from Poole Harbour and tissue weight in a) digestive gland (DG), and b) whole tissue (WT) across seasons in 2015.

(dry weight) limits for Pb and Cd, respectively. The highest mean concentration of Pb was found in *R. philippinarum* from Holes Bay (north) at 4.34 µg/g (maximum of 5.37 µg/g). The highest mean concentration of Cd was found in *R. philippinarum* from the Wareham Channel at 1 µg/g (maximum of 1.28 µg/g). Therefore, concentrations of these metals found in *R. philippinarum* in Poole Harbour are below limits deemed safe for human consumption. Additionally, this indicates that the magnitude of metal pollution in Poole Harbour is not severe, and therefore suggests it may not be high enough to induce MT reliably. This may be what is occurring in the Wareham Channel, which exhibited insignificant relationships with metal and MT concentrations in *R. philippinarum*. For comparison, the magnitude of metal pollution in the Fal Estuary, in the south-west of the UK, which received mining waste in 1989, is much higher. Sediment metal concentrations in the most polluted part of the estuary, Restronguet Creek, are approximately two orders of magnitude higher than Poole Harbour for As and Cu, and two times more concentrated in Fe (Langston et al., 2003a).

During winter, the pattern of tissue concentrations of most metals in *R. philippinarum* across sites seems to be in accordance with sediment metal concentrations. This is also true of Cu, Zn, Ag, Sn, and Pb throughout each season, suggesting that the bioavailabilities of these metals to clams are relatively unaffected seasonally. However, Cr, Fe, Ni, As, and Cd vary in tissue concentration in summer and autumn seasons, suggesting the bioavailability of these metals to clams change seasonally. An explanation for this may be that the speciations of some metals (Cd, Cr, As) are particularly sensitive to redox potential (Bryan and Langston, 1992). They tend to be complexed with sulphide minerals in anoxic conditions, which have very low solubility (Morse, 1994). Moreover, reduced dissolved oxygen concentrations in summer due to eutrophic conditions may reduce the oxidation of metals from Fe and Mn oxides, further reducing metal bioavailability (Mubiana et al., 2005). This may explain the generally lower As, Cr and Cd tissue concentrations in *R. philippinarum* in summer, as eutrophic conditions deplete dissolved oxygen in the water column (Humphreys et al., 2007). Cd bioavailability can increase with lower salinity due to lower complexation with the ion chloride (Ivankovic et al., 2005; Langston et al., 1998; Smaoui-Damak et al., 2009). This may explain the higher Cd accumulation in the Wareham Channel compared to other sites, as the salinity was lower. Furthermore, Ng and Wang (2004) have reported that the interaction of the metals could affect their uptake, accumulation, and toxicity by aquatic organisms.

### 4.2. Biotic seasonal effects to MT

Metal exposure seems to have a large influence on MT induction during winter, evidenced by highest MT concentrations in Holes Bay (north) and significant positive relationships between tissue metal and MT concentrations in *R. philippinarum*. However during spring, summer, and (to some extent) autumn, relationships between tissue metal and MT concentrations break down. Moschino et al. (2012) reported similar findings when assessing MT response during summer and autumn in the Venice lagoon, Italy, suggesting a cautionary

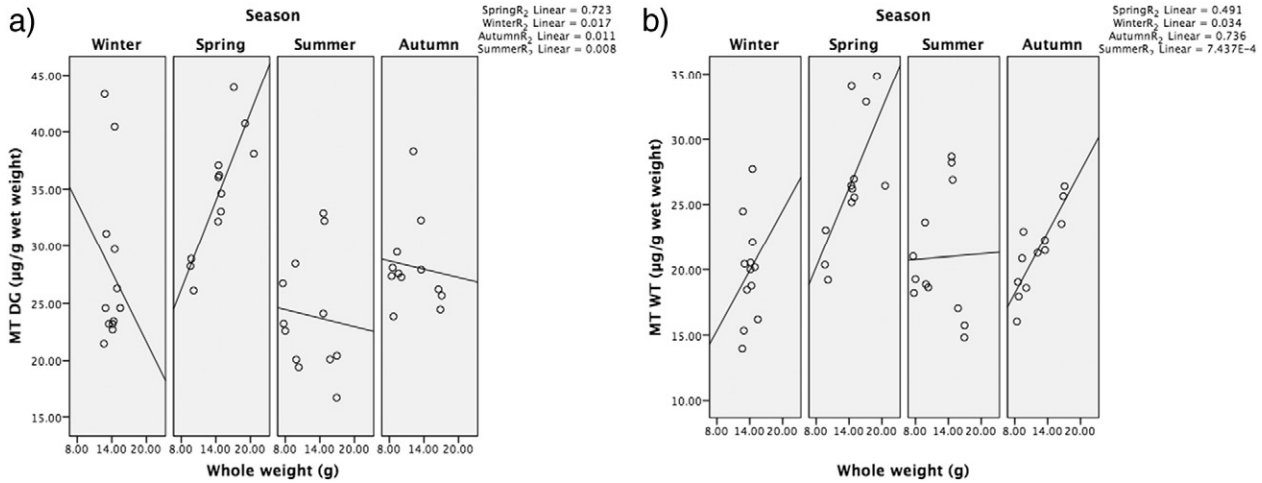


**Fig. 5.** Linear regression between MT concentrations in a) digestive gland (DG), and b) whole tissue (WT) in *R. philippinarum* from Poole Harbour and whole weight across seasons in 2015.

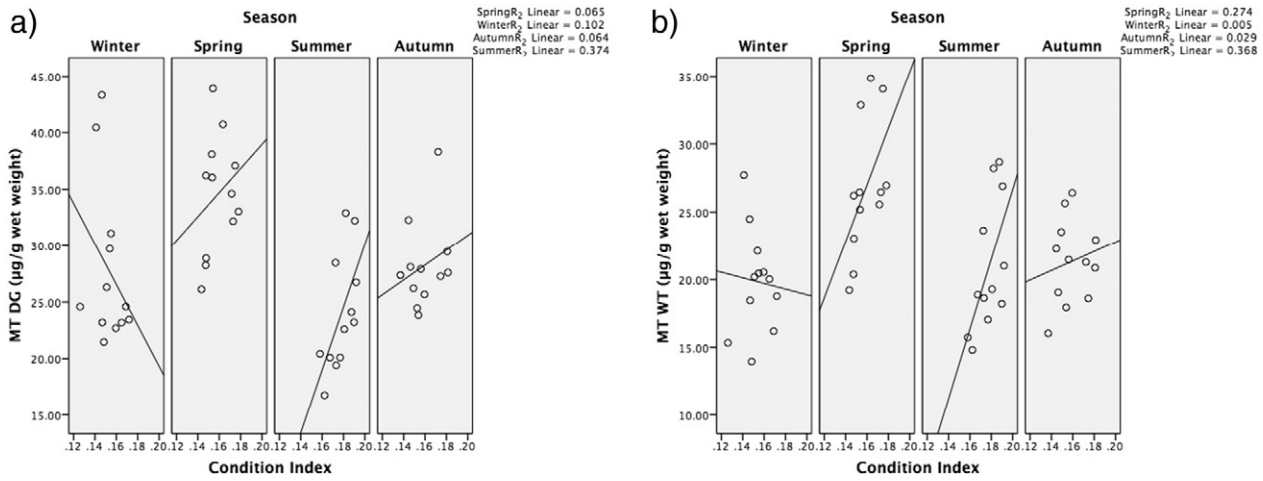


**Fig. 6.** Linear regression between MT concentrations in a) digestive gland (DG), and b) whole tissue (WT) in *R. philippinarum* from Poole Harbour and condition index across seasons in 2015.

approach to using *R. philippinarum* for biomonitoring. Other physiological processes such as mass increase, condition, and gametogenesis appear to override the effect of metal exposure on MT concentration. Some studies on mussels (*Mytilus galloprovincialis*) show MT concentrations to decrease during spring periods, which coincide with maximum digestive gland mass (Raspor et al., 2005; Raspor et al., 2004). This is due to gametogenesis, where the gonads develop and encroach on digestive gland tissue. This swells the digestive gland and dilutes the concentration of MT. Clams have similar physiology to mussels, in that gonadal tissue is indiscrete from digestive gland tissue in clams. However, the present study shows significant positive relationships between digestive gland tissue weight and MT concentrations in spring. In addition, maximum MT concentrations occurred in spring, when digestive gland weight was not at a minimum. Therefore, the data presented here suggests that biological dilution is not causing MT concentrations to differ independently of metal exposure.

Maximum concentrations of MT have been reported to occur in spring in *R. philippinarum* (Bocchetti et al., 2008). Some studies suggest MT is highest during gametogenesis and decreases after spawning (Paul-Pont et al., 2010). This is likely due to the onset of gametogenesis, and the associated hormonal secretion that can induce MT (Baudrimont et al., 2006; Baudrimont et al., 1997; Mao et al., 2012). Furthermore, a study by Meistertzheim et al. (2009) found MT in gonadal tissue to be in high concentrations in *Crassostrea gigas*. It suggests that oocytes within the gonads produce MT, which provides a protective role against metals (and other harmful agents) in a variable environment during embryo-larval stages, and that MTs may also play a role during meiosis. Consequently, as gonads develop, MT concentrations can increase. *R. philippinarum* in Poole Harbour undergo gametogenesis during spring, and regularly exhibit two spawning events, given the right thermal conditions, in around June and October (Humphreys et al., 2007). Therefore, it is probable that the increase in MT in spring in the present study is related to gametogenesis and the development of the gonads.

Although tissue concentrations for metals stayed relatively constant seasonally, metal body burden (tissue concentrations multiplied by tissue weight) increased during spring, concurrently with tissue weight. This is likely due to increased metabolic activity and increased food uptake to support growth, leading to increased dietary metal uptake (Rouane-Hacene et al., 2015). It can be argued that this caused MT concentrations to increase in spring, rather than gametogenesis. However, there were insignificant relationships between MT concentrations and metal body burden during spring. Therefore, it is still more likely that tissue weight (related to metal body burden, but a proxy for gametogenesis) is causing MT response to be independent of metal exposure, from spring onwards.

MT concentrations demonstrated a significant positive relationship with condition index in summer (Fig. 9). In summer, energy expended during gametogenesis may cause the health condition of clams to be poor, possibly causing mortality (Meistertzheim et al., 2009). This could reduce MT induction as energy resources are depleted. Increased temperatures of 25 °C and anoxic conditions due to extensive algal cover caused mortality of *R. philippinarum* during summer in Poole Harbour in 2003 (Humphreys et al., 2007). It is therefore possible that the relatively poor physical condition of organisms (and by proxy weight) is reducing the capability of MT induction in summer.

The number of significant relationships between MT and metal concentrations are similar for digestive gland and whole tissue, during winter, but lower in gill tissue (Table 1). However, the strength of relationships were moderate to strong between MT and certain metals in gills. One reason for this may be that gills are in direct contact with the surrounding environment, and reflect short-term contaminant exposure and are the primary target for water contaminants (Bebianno et al., 2004; Cravo et al., 2013). Nevertheless, other studies advocate the use of MT in the digestive gland in *Ruditapes decussatus* as the most appropriate biomarker of metal pollution (Smaoui-Damak et al., 2009). The present study shows the most moderate to strong significant

**Table 4**
Abiotic variables measured during sampling at each site (Note: W is winter, Sp is spring, Su is summer, A is autumn, DO is dissolved oxygen).

| | Wareham Channel | | | | Arne Bay | | | | Holes Bay | | | | Holes Bay (north) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | W | Sp | Su | A | W | Sp | Su | A | W | Sp | Su | A | W | Sp | Su | A |
| Temp (°C) | 7.85 | 12.8 | 18.3 | 11.6 | 3.97 | 12.9 | 18.8 | 12.7 | 4.90 | 13.4 | 19.2 | 13.1 | 8.65 | 14.9 | 19.2 | 14.4 |
| pH | 8.45 | 8.7 | 8.11 | 8.27 | 8.7 | 8.7 | 8.31 | 8.15 | 8.7 | 8.65 | 8.04 | 8.36 | 8.4 | 8.5 | 8.08 | 8.50 |
| Salinity (ppt) | 19.0 | 20.0 | – | 20.1 | 27.3 | 21.2 | – | 26.9 | 27.1 | 21.8 | – | 30 | 24 | 20.2 | – | 21.6 |
| DO (mg/l) | 9.50 | 7.05 | – | 7.95 | 7.30 | 7.40 | – | 8.79 | 7.56 | 6.28 | – | 8.29 | 7.01 | 7.22 | – | 8.37 |

**Table 5**
Linear regression analysis between MT in the digestive gland (DG), gills (G), and whole tissue (WT) and abiotic variables at each site across seasons (Note: DO is dissolved oxygen, +ve is positive relationship, −ve is negative relationship).

| | | Wareham Channel | | | Arne Bay | | | Holes Bay | | | Holes Bay (north) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DG | G | WT | DG | G | WT | DG | G | WT | DG | G | WT |
| Temp (°C) | $R^2$ | – | – | – | – | 0.471 +ve | 0.385 +ve | – | – | 0.346 +ve | 0.592 −ve | 0.532 −ve | 0.452 −ve |
| | P | – | – | – | – | 0.014 | 0.031 | – | – | 0.044 | 0.003 | 0.007 | 0.017 |
| pH | $R^2$ | 0.572 +ve | 0.337 +ve | 0.508 +ve | – | 0.465 −ve | – | – | – | – | 0.390 +ve | – | 0.866 +ve |
| | P | 0.008 | 0.048 | 0.009 | – | 0.015 | – | – | – | – | 0.03 | – | <0.001 |
| Salinity (ppt) | $R^2$ | – | – | – | – | – | – | – | – | – | – | – | – |
| | P | – | – | – | – | – | – | – | – | – | – | – | – |
| DO (mg/l) | $R^2$ | – | – | – | – | – | – | – | – | 0.671 −ve | – | – | – |
| | P | – | – | – | – | – | – | – | – | 0.007 | – | – | – |

relationships in whole tissue between MT and metals during winter (Table 1). Therefore whole tissue MT may be advantageous to use as a biomarker due to the simplicity of dissection.

### 4.3. Abiotic seasonal effects to MT

Consistent relationships between MT concentration and other abiotic variables such as pH, salinity, and dissolved oxygen were not found across individual sites. It is important to note that these variables were measured at each sampling, and not throughout the year, so may not be representative. However, this may suggest abiotic factors do not influence MT concentrations in the same manner, but have specific localised effects. For example, organisms subjected to chronic metal exposure, or parasitic infections may have adapted detoxification mechanisms (Paul-Pont et al., 2010). Smaoui-Damak et al. (2004) showed that location was an important contributor to MT concentrations rather than metal concentrations in *R. decussatus*. This may be due to variations in environmental conditions, reproductive process, or genetic factors (Tanguy et al., 2003). In this study, minimum concentrations of MT were found in Holes Bay (north) in summer, which exhibited a significant negative relationship with temperature. This could be due to increased temperature and light irradiance reducing total oxyradical scavenging capacities (Bocchetti et al., 2008). This could be exacerbated by extreme temperature fluctuations as benthic habitats are exposed to air at low water at this site. However, negative relationships with temperature and MT concentrations were not evident in *R. philippinarum* from other sites.

## 5. Conclusion

In order for MT to present a useful tool in biomonitoring and relay the biological impact of metals to *R. philippinarum*, external seasonal influences to MT concentration must be constrained. Here it is shown that gametogenesis and the associated increase in MT concentrations, and condition index, override the effect on MT concentration by metal exposure. It is consequently not a reliable biomarker for metal pollution during spring and summer, when clams are undergoing reproductive processes and may be in relatively poor body condition. It is therefore recommended that MT is only used as a biomonitoring tool during winter periods, when *R. philippinarum* is at a resting reproductive state. This study adds to the knowledge of *R. philippinarum* MT response at latitudes approaching the limit of its geographic extent, and constrains its potential as a cosmopolitan bioindicator species. In addition, the concentration of metals in *R. philippinarum* from the most polluted site in Poole Harbour, Holes Bay (north), is unlikely to pose a risk to human health following consumption.

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